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㉖ Encapsulated nucleic acids.

㉗ RNA and/or DNA, e.g. poly(I:C) or anti-sense
RNA, is encapsulated as microcapsules in a biodeg-
radable, biocompatible polymeric material, for the
controlled release of the nucleic acid on administra-
tion and the induction of interferon production.

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ENCAPSULATED NUCLEIC ACIDS

This invention relates to encapsulated nucleic acids.

Interferon is a potential inhibitor of viral replication. It is a host-specific protein naturally secreted by organisms exposed to viral agents, see Isaacs, A. Advan. Virus Res. 10,1(1963) and Ho, M. in "Interferons" (N.B.Finter, ed.), Chapter 2, pp 21-54 North-Holland Publ., Amsterdam (1967). More specifically, after exposure to viral agents, organisms synthesize interferon from messenger RNA transcribed from a host gene; see Wagner, R.R., Huang, A.S. Virology 28, 1 (1963); Heller, E. Virology 21, 652 (1963) and Wagner, R. R. Trans. Ass. Am. Physicians 76, 92 (1963). Interferon is species specific, but biologically active against a variety of viruses; see Buckler, C.E., Baron, S. J. Bacteriol 91, 231 (1966) and Merigan, T.C. Science 145, 811 (1964).

Because of the potential therapeutic value of protecting an animal from a variety of viral infections by the animal's own interferon system, there has been research concentrated on finding non-viral agents capable of inducing the production of interferon; see Ho, M., Postic, B., Ke, Y.H. in "Ciba Foundation Symposium on Interferon" (Woistenholme and O'Connor, ed.), p. 19, Little, Brown, Boston, Mass., USA (1967) and Ho, M., Fantes, K.H., Burke, D.C., Frater, N.B. In "Interferons" supra p.181. In particular, a complex of polyribonucleosinic-polyribocytidylic acid, also known as poly(I:C), has been shown to be a potent inducer of interferon when administered in low doses; see Field, A.K., Tytell, A. A., Lampson, G.P. Hilleman, M.R. Proc. Nat. Acad. Sci. 58, 1004-1010 (1967); Parks J.H., Baron, S. Science 162, 811-813 (1968); Wothington, M., Levy, H., Rice, J. Proc. Soc. Exp. Biol. Med. 143, 638-643 (1973); Zeleznick, L.D., Bhuyan, B.K. Proc. Soc. Exp. Biol. Med. 130, 126-128 (1969); Levy, H.B., Law, L.W., Rabson, A.S. Proc. Nat. Acad. Sci. 62, 357-361 (1969) and Gelboin, H., Levy, H.B. Science 167, 205-207 (1970).

Major disadvantages associated with the use of poly(I:C) to induce interferon production include its toxicity at high doses and its short half-life in humans. The short half-life is due to the fact that human serum contains a high concentration of a nucleolytic enzyme complex that rapidly hydrolyzes, and thereby inactivates, poly(I:C); see Nordlund, J.J., Wolff, S.M., Levy, H.B. Proc. Soc. Exp. Biol. Med. 133, 439-444 (1970). To overcome the problems associated with poly(I:C) administration, most researchers have developed multiple dosing schedules that require repetitive injections of small quantities of poly(I:C) for periods of four to

seven days; see Kend's, M. J. Biol. Response Mod. 4, 503-511 (1985); Stephen, E.L., Bammons, M.L., Pannier, W.L., Barron, S., Spertzel, F.O., Levy, H.G. J. Infect. Dis. 136, 122-126 (1977); Levy, H.B., Baer, G., Baron, S., et al. J. Infect. Dis. 132, 434-439 (1975) and Sammons, M.L., Stephen, E.L., Levy, H.B., Baron, S., Himas, D.E. Antimicrob. Agents Chemother. 11, 80-83 (1977).

RNA also has a short half-life in the body; see Klausner, A. BioTechnology 3, 763-764 (1985) and Izant, J.G., Weintraub, H. Science 229, 345-352 (1985).

According to the present invention, controlled-release microcapsules comprise RNA or DNA, or a mixture thereof, encapsulated in a biocompatible, biodegradable polymer. After a single administration, particularly by injection, e.g. parenterally, the microcapsules release the RNA or DNA at a controlled rate, resulting in induction of high titers of interferon. The microcapsules protect the nucleic acid from enzymatic digestion or degradation before it is released.

The present invention provides a pharmaceutical composition designed for the controlled release or sustained release of an effective amount of RNA and/or DNA, over an extended period of time; the RNA or DNA is encapsulated in a biocompatible, biodegradable polymeric material which protects the nucleic acid from enzymatic degradation. The microcapsules can have an internal structure whereby a single reservoir of RNA or DNA is coated with a polymeric membrane; alternatively, the microcapsules can have an internal structure whereby the RNA or DNA is dispersed within a polymeric matrix.

The term biocompatible may be defined as non-toxic to the human body, non-carcinogenic and non-inflammatory in body tissues. The matrix material should be biodegradable in the sense that the polymeric material should degrade by bodily processes to products readily disposable by the body, and should not accumulate in the body. The biodegraded products also should be biocompatible with the body in the sense that the polymeric matrix is compatible with the body.

A particular application for the controlled release of RNA pertains to the delivery of anti-sense RNA to the body, to turn off the expression of specific genes. It has been shown that gene expression can be turned off at the translation level. More specifically, translation can be blocked by introducing into cells RNA that is complementary to a particular message RNA produced in that cell. The complementary RNA (anti-sense RNA) will hybridize with the message RNA and prevent the

synthesis of the protein coded on the message RNA. Because of the short half-life of RNA in the body, a practical way of delivering anti-sense RNA for therapeutic treatment is by releasing it from biocompatible, biodegradable microcapsules.

The excipient for the nucleic acid microcapsules can be a synthetic polymer. Examples of the polymeric excipients suitable for use in the present invention include poly(D,L-lactide), poly(glycolic acid), copolymers of mixed D,L-lactide and glycolide, copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides) and poly(beta-hydroxybutyric acid).

The nucleic acid can be a synthetic or naturally-produced species. The RNA and/or DNA may be either single-stranded or double-stranded.

Microcapsules of the present invention can be prepared by any method which is capable of producing microparticles in a size and range acceptable for use in an injectable composition. The microcapsules can be loaded with from 0.01 to 75 wt.% of the RNA or DNA, based on the polymeric matrix. The microcapsules may range in size from 0.1 to 500 μm . In the treatment of animals, the microcapsules of the present invention are dispersed in a pharmaceutically acceptable carrier suitable for parenteral administration. One skilled in the art is well aware of the dosages of RNA or DNA required to induce the production of interferon in animals and to aid protection from a variety of viral infections.

The following Example illustrates the preparation of poly(I:C) microcapsules comprising a poly(DL-lactide-co-glycolide) (DL-PLG) excipient.

Example

A DL-PLG polymer solution is first prepared by introducing 1.0 g of DL-PLG and 50 g of ethyl acetate (EM Science, Gibbstown, N.J., U.S.A.) into a 4 oz (115 g) glass jar. The DL-PLG has a 53:47 mole ratio of lactide-to-glycolide and an inherent viscosity of 0.91 dL/g as measured at 30°C in hexafluoroisopropanol at a concentration of 0.15 g/dL. A 25 mm Teflon (registered Trade Mark) stir bar is put into the jar and the jar is then sealed with a Teflon-lined scrap cap. The bottle is placed on a hot plate/stirrer. The DL-PLG/ethyl acetate mixture is stirred while heating to 58°C to dissolve the polymer. Once the polymer is dissolved, the jar is removed from the hot plate/stirrer and the DL-PLG solution is allowed to cool to 45°C. Next, 7.5 g of the DL-PLG solution are transferred to a Pyrex test tube (150 mm long, 17 mm outer diameter) containing 100 mg of poly(I:C) (Double strand, physiological salt, lyophilized, Pharmacia/PL Biochemicals, Piscataway, N.J., U.S.A.).

The remainder of the DL-PLG solution is transferred to a 100-ml resin kettle (Knotes Glass Co., Vineland, N.J., U.S.A.) filled with a true-bore stir-shaft, a 38-mm Teflon-turbine impellor (Ace Glass, Inc., Vineland, N.J., U.S.A.), and a Fisher Stead-speed motor (Fisher Scientific Company, Norcross, Ga, U.S.A.). The solution containing the poly(I:C) is then homogenized at a setting of 5.5 with a Brinkmann Polytron (Brinkmann Instruments, Model PT-10-35 with a PTIOST probe, from Westbury, N.Y., U.S.A.) for four 1-min. bursts with 30 sec. between each burst. The resulting polymer/poly(I:C) suspension is then added to the resin kettle and stirred at 1200 rpm (reverse position). Next, 14 ml of silicone oil (Dow Corning 200 Fluid, 100 C St. Midland, N.J. U.S.A.) are pumped into the resin kettle at a rate of 2 ml/min. After the silicone oil addition is complete, the contents of the resin kettle are quickly poured into 3 litres of heptane (Phillips Chemical Co., Borger, Tx., U.S.A.) contained in a 4-litre beaker and stirred at about 750 rpm with a 50 mm stainless steel impellor. The microcapsules are stirred in the heptane for 45 min. to extract any remaining ethyl acetate. The microcapsules are then collected by vacuum filtration, washed with about 250 ml of heptane, and dried in a vacuum chamber maintained at room temperature.

Microcapsules made by the above process contained 0.55 wt % poly(I:C).

The *in vitro* release characteristics of these microcapsules were measured. The accompanying drawing shows the *in vitro* release curves of poly(I:C) in unencapsulated form and from the microcapsules into a receiving fluid consisting of 0.15 M sodium chloride maintained at 37°C. The amount of poly(I:C) released into the sodium chloride solution was quantified spectrophotometrically at 250 nm. Compared to unencapsulated poly(I:C), the microencapsulated poly(I:C) is released into the aqueous receiving fluid in a controlled manner over a longer period of time. The microcapsules release the poly(I:C) *in vitro* for up to 118 hours.

Claims

1. A composition which comprises RNA and/or DNA encapsulated as microcapsules in a biodegradable, biocompatible polymeric material.
2. A composition according to claim 1, wherein the RNA and/or DNA is RNA.
3. A composition according to claim 1, wherein the RNA and/or DNA is a complex of polyriboinosinic-polyribocytidylic acid.
4. A composition according to any preceding claim, which comprises from 0.01 to 75 wt. % of the RNA and/or DNA based on the polymeric material.

5. A composition according to any of claims 1 to 4, wherein the RNA and/or DNA is double-stranded.

6. A composition according to any of claims 1 to 4, wherein the RNA and/or DNA is single-stranded.

7. A composition according to any preceding claim, wherein the polymer is selected from poly-(D,L-lactide), poly(glycolic acid), copolymers of mixed D,L-lactide and glycolide, copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides) and poly(beta-hydroxy butyric acid).

8. A composition according to claim 3, wherein the polymer is poly(DL-lactide-co-glycolide).

9. A composition according to any preceding claim, which comprises a pharmaceutically acceptable carrier for the microcapsules, the carrier being suitable for parenteral administration to an animal.

10. The use of a composition according to any preceding claim for the manufacture of a medicament for use in protecting an animal from viral infections, the RNA and/or DNA being released at a controlled, predetermined rate to induce the production of interferon and thereby stimulate the immune system of the animal.

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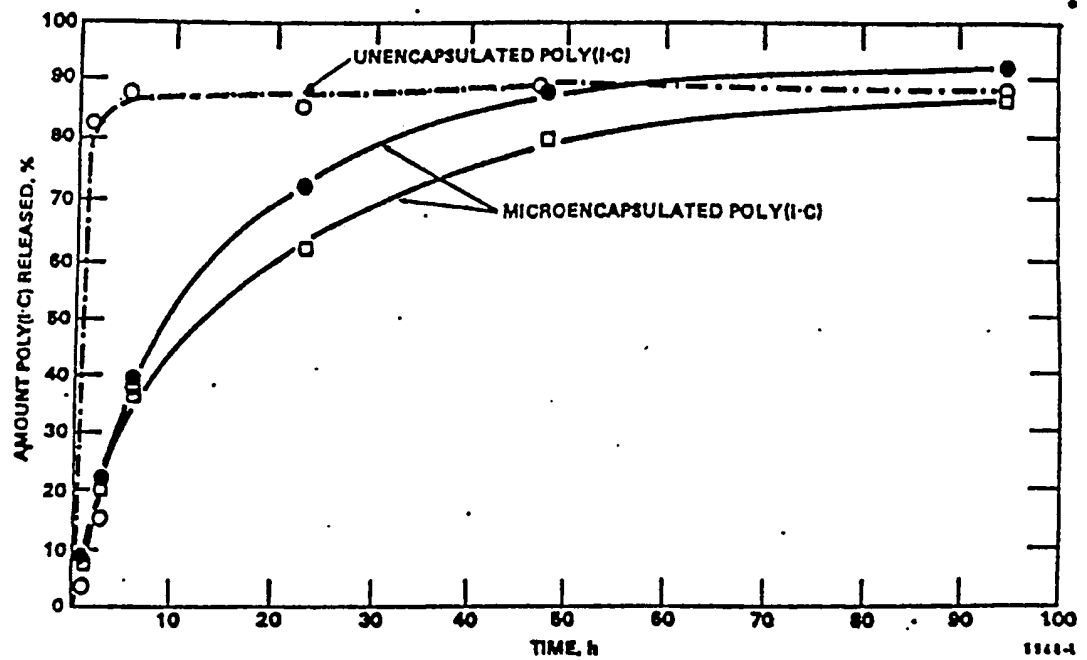
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European Patent
Office

EUROPEAN SEARCH REPORT

Application Number

EP 87 30 3955

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
Y	US-A-4 124 702 (G.P. LAMPSON) * Whole document *	1-10	A 61 K 9/50 A 61 K 9/16 A 61 K 31/70
Y	CHEMICAL ABSTRACTS, vol. 105, no. 20, 17th November 1986, page 371, abstract no. 178326k; D.A. EPPSTEIN et al.: "Pathways to increasing efficacy of interferons: drug synergy and sustained-release", & BIOL. INTERFERON SYST. PROC, TNO-ISIR MEET. INTERFERON SYST., 4TH 1985 (PUB. 1986), 401-9 * Whole abstract *	1-10	
A	US-A-3 679 654 (R. MAES) * Whole document *	1-10	
A	GB-A-2 067 201 (GOVERNMENT OF THE UNITED STATES OF AMERICA) * Page 1, lines 3-5,20-46; claims *	1-10	
A	JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 73, no. 9, September 1984, pages 1294-1297, American Pharmaceutical Association, Easton, PA, US; L.M. SANDERS et al.: "Controlled release of a luteinizing hormone-releasing hormone analogue from poly(d,l-lactide-co-glycolide) microspheres" * Page 1294; pages 1296-1297, chapter "results and discussion" *	7,8	TECHNICAL FIELDS SEARCHED (Int. Cl. 4) A 61 K C 07 H
A	EP-A-0 058 481 (IMPERIAL CHEMICAL INDUSTRIES PLC) * Claims *	7,8	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 21-07-1988	Examiner MUELLNERS W.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			